aladdin®

Protein A/G Magnetic Beads

P751576

Store at 2-8℃ (24 months). Upon delivery aliquot.

Introduction:

Protein A/G Magnetic Beads are 200 nm nanoscale magnetic beads with a large amount of Protein A/G covalently bound to their surface. The nanoscale size of the beads provides an extremely high surface area, resulting in more binding sites. This means less magnetic bead usage is required, and non-specific binding is minimized. The recombinant Protein A/G contains five immunoglobulin-binding domains from Protein A and two binding domains from Protein G, significantly enhancing binding capacity compared to single Protein A or Protein G. This product is widely used for immunoprecipitation (IP) or co-immunoprecipitation (Co-IP) of antigens from cell lysates, cell culture supernatants, serum, ascites, and other samples. The magnetic separation feature allows each IP and Co-IP procedure to save up to 40% of the time.

Usage method:

Adherent Cell Samples:

1. Remove the culture medium and wash the cells twice with PBS.
2. Collect the cells into a 1.5 mL EP tube, add IP Lysis/Wash Buffer in proportion, and include inhibitors such as PMSF. Mix well and place on ice for 5-20 minutes (mixing several times during this period).
3. Centrifuge at 4°C, 12000-16000×g, for 10 minutes to collect the supernatant. Keep the supernatant on ice for subsequent experiments (or store at -80°C for long-term storage).

Suspension Cell Samples:

1. Centrifuge the cells at 4°C, 500-1000×g, for 10 minutes, and discard the supernatant.
2. Wash the cells once with PBS by resuspending the cell pellet in PBS, then centrifuge again at 4°C, 500-1000×g, for 5 minutes, and discard the supernatant.
3. Resuspend the cells in pre-cooled IP Lysis/Wash Buffer. Use 500 µL of IP Lysis/Wash Buffer for every 50 mg of cells. Add inhibitors such as PMSF, mix well, and place on ice for 5-20 minutes (mixing several times during this period).
4. Centrifuge at 4°C, 12000-16000×g, for 10 minutes to collect the supernatant. Keep the supernatant on ice for subsequent experiments (or store at -80°C for long-term storage).

Serum Samples:

It is generally recommended to dilute serum samples with IP Lysis/Wash Buffer to achieve a final protein concentration of 50-150 µg/mL. Keep the diluted samples on ice (or store at -20°C for long-term storage).

Preparation of Immune Complexes:

Note: The amount of sample required and the incubation time depend on the specific antibody-

antigen system and may need optimization to achieve maximum yield.

The following protocol is designed for 2-10 μg of affinity-purified antibody and can be scaled up as needed.

1. In a centrifuge tube, combine the cell lysate with 2-10 μg of immunoprecipitation antibody for each sample. The recommended total protein amount for each immunoprecipitation reaction is 500-1500 μg.
2. Dilute the antibody and prepared sample to 300-500 μL with IP Lysis/Wash Buffer.
3. Incubate at room temperature for 1-2 hours, or at 4°C for 2-4 hours to form immune complexes.

Immunoprecipitation:

Note: To ensure even distribution of the magnetic beads, mix the beads thoroughly by repeated inversion or gentle vortexing before use.

1. Add 20-50 μL of Protein A/G Magnetic Beads to a 1.5 mL centrifuge tube.
2. Add 500 μL of pre-cooled PBS to the beads and mix gently.
3. Place the centrifuge tube on a magnetic rack to collect the beads on one side of the tube. Remove the supernatant.
4. Add 200-500 μL of IP Lysis/Wash Buffer to the tube. Mix by inverting the tube several times or gentle vortexing for 1 minute. Collect the beads using the magnetic rack and remove the supernatant.
5. Add the antigen sample/antibody mixture to the tube containing the beads. Keep the mixture homogenous and incubate at room temperature for 1-2 hours, or at 4°C for 2-4 hours.
6. Collect the beads using the magnetic rack, remove the unbound sample, and save it for analysis.
7. Add 1000 μL of IP Lysis/Wash Buffer to the tube, mix the beads gently for 5-10 minutes. Collect the beads and discard the supernatant. Repeat the wash twice more.
8. Denaturing Elution: Add 80-100 μL of SDS-PAGE Sample Loading Buffer (1×) to the tube. Heat the sample in a water bath or metal bath at 100°C for 10 minutes. Separate the beads using the magnetic rack and retain the supernatant containing the target antigen.

Note: If protein activity needs to be maintained, the following elution method can be used.

Low pH Elution: Add 100 μL of Elution Buffer to the tube. Mix well and incubate at room temperature for 5-10 minutes. Separate the beads using the magnetic rack and retain the supernatant containing the target antigen. Neutralize the low pH by adding 20 μL of Neutralization Buffer to every 100 μL of eluate.

Matters needing attention:

1. Please read the instruction manual carefully before performing the experiment.
2. Do not centrifuge at high speed, dry, or freeze the magnetic beads, as these operations can cause bead aggregation and reduce binding capacity.
3. Different types of antibodies have varying affinities for antigens in IP experiments, and the binding can also be affected by the IP Lysis/Wash Buffer. Therefore, you may optimize the

operational details or select and formulate the buffer for your experiment.

4. Mix the magnetic beads thoroughly before use. Store the beads in their storage solution to prevent drying.
5. This product is for research use only.

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